

NTP TECHNICAL REPORT
ON THE
TOXICOLOGY AND CARCINOGENESIS
STUDIES OF PRIMIDONE
(CAS NO. 125-33-7)
IN F344/N RATS AND B6C3F₁ MICE
(FEED STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

September 2000

NTP TR 476

NIH Publication No. 00-3966



National Toxicology Program

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

Listings of all published NTP reports and ongoing studies are available from NTP Central Data Management, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). The Abstracts and other study information for 2-year studies are also available at the NTP's World Wide Web site: <http://ntp-server.niehs.nih.gov>.

NTP TECHNICAL REPORT
ON THE
TOXICOLOGY AND CARCINOGENESIS
STUDIES OF PRIMIDONE
(CAS NO. 125-33-7)
IN F344/N RATS AND B6C3F₁ MICE
(FEED STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

September 2000

NTP TR 476

NIH Publication No. 00-3966



National Toxicology Program

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

CONTRIBUTORS

National Toxicology Program

Evaluated and interpreted results and reported findings

J.K. Dunnick, Ph.D., Study Scientist
D.A. Bridge, B.S.
J.R. Bucher, Ph.D.
J.K. Haseman, Ph.D.
J. Heindel, Ph.D.
R.A. Herbert, D.V.M., Ph.D.
J.R. Leininger, D.V.M., Ph.D.
R.R. Maronpot, D.V.M.
D.P. Orzech, M.S.
A. Radovsky, D.V.M., Ph.D.
G.N. Rao, D.V.M., Ph.D.
J.H. Roycroft, Ph.D.
C.S. Smith, Ph.D.
G.S. Travlos, D.V.M.
D.B. Walters, Ph.D.
K.L. Witt, M.S., Integrated Laboratory Systems, Inc.

Battelle Columbus Laboratories

Conducted studies, evaluated pathology findings

P.J. Kurtz, Ph.D., Principal Investigator
M.R. Hejtmancik, Ph.D.
J.D. Johnson, Ph.D.
R.L. Persing, D.V.M.
J.D. Toft, II, M.S., D.V.M.

Experimental Pathology Laboratories, Inc.

Provided pathology quality assurance

J.F. Hardisty, D.V.M., Principal Investigator
S. Botts, M.S., D.V.M., Ph.D.
E.T. Gaillard, M.S., D.V.M.

Dynamac Corporation

Prepared quality assurance audits

S. Brecher, Ph.D., Principal Investigator

Analytical Sciences, Inc.

Provided statistical analyses

R.W. Morris, M.S., Principal Investigator
N.G. Mintz, B.S.
S. Rosenblum, M.S.

NTP Pathology Working Group

*Evaluated slides, prepared pathology report on rats
(18 July 1996)*

D.G. Goodman, V.M.D., Chairperson
PATHCO, Inc.
S. Botts, M.S., D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
J. Cullen, V.M.D., Ph.D.
North Carolina State University
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
J.R. Leininger, D.V.M., Ph.D.
National Toxicology Program
C. Merrill, D.V.M., Observer
North Carolina State University
A. Nyska, D.V.M.
National Toxicology Program
A. Radovsky, D.V.M., Ph.D.
National Toxicology Program

*Evaluated slides, prepared pathology report on mice
(17 July 1996)*

P.K. Hildebrandt, D.V.M., Chairperson
PATHCO, Inc.
R. Cattley, V.M.D., Ph.D.
Chemical Industry Institute of Toxicology
E.T. Gaillard, M.S., D.V.M.
Experimental Pathology Laboratories, Inc.
J.R. Hailey, D.V.M.
National Toxicology Program
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
J.R. Leininger, D.V.M., Ph.D.
National Toxicology Program
A. Nyska, D.V.M.
National Toxicology Program
A. Radovsky, D.V.M., Ph.D.
National Toxicology Program

Biotechnical Services, Inc.

Prepared Technical Report

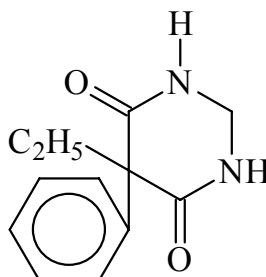
S.R. Gunnels, M.A., Principal Investigator
L.M. Harper, B.S.
D.C. Serbus, Ph.D.
J.E. Marshall, M.S.
S.M. Swift, B.S.

CONTENTS

ABSTRACT	5
EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY	11
TECHNICAL REPORTS REVIEW SUBCOMMITTEE	12
SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS	13
INTRODUCTION	15
MATERIALS AND METHODS	25
RESULTS	35
DISCUSSION AND CONCLUSIONS	63
REFERENCES	69
APPENDIX A	Summary of Lesions in Male Rats in the 2-Year Feed Study of Primidone
	81
APPENDIX B	Summary of Lesions in Female Rats in the 2-Year Feed Study of Primidone
	117
APPENDIX C	Summary of Lesions in Male Mice in the 2-Year Feed Study of Primidone
	147
APPENDIX D	Summary of Lesions in Female Mice in the 2-Year Feed Study of Primidone
	177
APPENDIX E	Genetic Toxicology
	209
APPENDIX F	Organ Weights and Organ-Weight-to-Body-Weight Ratios
	219
APPENDIX G	Hematology and Clinical Chemistry Results
	223
APPENDIX H	Determinations of Primidone and Phenobarbital in Plasma
	229
APPENDIX I	Reproductive Tissue Evaluations and Estrous Cycle Characterization
	235
APPENDIX J	Chemical Characterization and Dose Formulation Studies
	239
APPENDIX K	Feed and Compound Consumption in the 2-Year Feed Studies of Primidone
	251

APPENDIX L	Ingredients, Nutrient Composition, and Contaminant Levels in NIH-07 Rat and Mouse Ration	257
APPENDIX M	Sentinel Animal Program	261
APPENDIX N	Single-Dose Toxicokinetic Studies in F344/N Rats and B6C3F₁ Mice	265
APPENDIX O	Continuous Breeding Study in Swiss (CD-1[®]) Mice	279

ABSTRACT



PRIMIDONE

CAS No. 125-33-7

Chemical Formula: $C_{12}H_{14}N_2O_2$ Molecular Weight: 218.28

Synonyms: 5-Aethyl-5-phenyl-hexahydropyrimidin-4,6-dion; 2-deoxyphenobarbital; 2-desoxyphenobarbital; desoxyphenobarbitone; 5-ethylidihydro-5-phenyl-4,6 (1H,5H)-pyrimidinedione; 5-ethylhexahydro-4,6-dioxo-5-phenylphrimidine; 5-ethylhexahydro-5-phenylpyrimidine-4,6-dione; 5-ethyl-5-phenylhexahydropyrimidine-4,6-dione

Trade names: Cyral; Hexadiona; Hexamidine; Lepimidin; Lepsiral; Majsolin; Midone; Milepsin; Misodine; Misolyne; Mizodin; Mizolin; Mylepsin; Mylepsinum; Mysedon; Mysoline; Prilepsin; Primacione; Primaclone; Primacone; Primakton; Primadon; Prysoline; Pyrimidone; ROE 101; Sertan

Primidone is used alone or with other anticonvulsants in the control of grand mal, psychomotor, and focal epileptic seizures. It may control grand mal seizures refractory to other anticonvulsant therapy. Primidone was nominated by the National Cancer Institute for 2-year toxicology and carcinogenicity studies due to its human use as an anticonvulsant. Male and female F344/N rats and B6C3F₁ mice received primidone (greater than 99% pure) in feed for 14 days, 14 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, and mouse bone marrow cells.

14-DAY STUDY IN RATS

Five male and five female rats were exposed to 0, 1,250, 2,500, 5,000, 10,000 or 20,000 ppm primidone (equivalent to average daily doses of approximately 120, 240, 500, 970, or 1,100 mg primidone/kg body weight to males and 120, 240, 500, or 900 mg/kg to females) in feed for 14 days.

All 20,000 ppm females died before the end of the study as did one 10,000 ppm male and two 20,000 ppm males. The mean body weights of 10,000 ppm males and females and 20,000 ppm males were significantly less than those of the controls. Feed consumption by all exposed rats was generally similar to that by the controls. Males and females in the 10,000 and 20,000 ppm groups were observed to have eye discharge, ataxia, and abnormal posture and were thin and lethargic.

14-DAY STUDY IN MICE

Five male and five female mice were exposed to 0, 625, 1,250, 2,500, 5,000 or 10,000 ppm primidone (equivalent to average daily doses of approximately 100, 200, 400, or 800 mg/kg body weight to males and 100, 250, 500, or 900 mg/kg to females) in feed for 14 days. All mice in the 10,000 ppm groups and one male and one female mouse in the 5,000 ppm groups died on day 3 of the study. The mean body weights of mice in the 625, 1,250, 2,500, and

5,000 ppm groups were similar to those of the controls. Feed consumption by all exposed mice was generally similar to that by the controls. Males and females in the 10,000 ppm groups were observed to have abnormal posture, ataxia, and lethargy.

14-WEEK STUDY IN RATS

Groups of 10 male and 10 female rats were exposed to 0, 300, 600, 1,300, 2,500, or 5,000 ppm primidone (equivalent to average daily doses of approximately 20, 40, 100, 200, or 400 mg/kg) in feed for 14 weeks. All rats survived to the end of the study. The mean body weights of male and female rats in the 2,500 and 5,000 ppm groups were significantly less than those of the controls. Feed consumption by all exposed rats was generally similar to that by the controls.

A minimal to mild exposure-related thrombocytosis occurred on day 22 and at week 14 in all exposed groups of male rats and in females in the 1,300 ppm or greater groups. A minimal decrease in hemoglobin concentration occurred in 2,500 and 5,000 ppm male and female rats on day 22 and at week 14.

The incidences of centrilobular hepatocyte hypertrophy in male rats exposed to 600 ppm or greater and in female rats exposed to 1,300 ppm or greater were significantly greater than those in the controls. The severity of chronic nephropathy in male rats exposed to 1,300 ppm or greater increased with increasing exposure concentration.

14-WEEK STUDY IN MICE

Groups of 10 male and 10 female mice were exposed to 0, 300, 600, 1,300, 2,500, or 5,000 ppm primidone (equivalent to average daily doses of approximately 50, 100, 200, 400, or 1,000 mg/kg to males and 60, 120, 220, 440, or 1,100 mg/kg to females) in feed for 14 weeks. Three male and two female mice in the 5,000 ppm group died during week 1 of the study. The final mean body weights of all exposed groups were similar to those of the controls. Feed consumption by male mice in the 5,000 ppm group was slightly greater than that by the controls; this may have been due to feed spillage. Male and female mice in the 5,000 ppm groups were ataxic and lethargic.

Compared to controls, the estrous cycle lengths of females exposed to 1,300, 2,500, or 5,000 ppm were significantly longer. The liver weights of male and female mice exposed to 600 ppm or greater were significantly greater than those of the controls. The incidences of centrilobular hepatocyte hypertrophy in all exposed males and in females exposed to 600 ppm or greater and the incidences of cytoplasmic alteration of the adrenal gland and hematopoietic cell proliferation of the spleen in 2,500 and 5,000 ppm males and in 5,000 ppm females were significantly greater than in the controls.

2-YEAR STUDY IN RATS

Groups of 50 male and 50 female F344/N rats were exposed to 0, 600, 1,300, or 2,500 ppm primidone (equivalent to average daily doses of approximately 25, 50, or 100 mg/kg) in feed for 2 years.

Survival, Body Weights, and Feed Consumption

Survival of the 1,300 and 2,500 ppm males was significantly less than that of the controls. The mean body weights of males and females in the 2,500 ppm groups were less than those of the controls, beginning at week 29 for males and week 17 for females; the mean body weights of 1,300 ppm males and females were less than those of the controls during the second year of the study. Feed consumption by all exposed groups of rats was generally similar to that by the controls.

Pathology Findings

Male rats exposed to primidone had increased incidences of thyroid gland follicular cell neoplasms (adenoma and/or carcinoma). All exposed groups of male rats had follicular cell adenomas or carcinomas (combined) at incidences above the historical control range, with the highest incidence in the 1,300 ppm group.

Hepatocyte cytoplasmic vacuolation and centrilobular hypertrophy were associated with primidone exposure in male and female rats. These changes were more severe in females than in males and the incidences in all exposed groups of females were significantly greater than those in the controls. Females in the 2,500 ppm group had an increased incidence of hepatocellular eosinophilic foci.

In 2,500 ppm males, the incidence of renal tubule hyperplasia was greater than that in the controls in the standard evaluation. Additional hyperplasias were found in the extended evaluation, and the incidences in exposed groups of males were significantly greater than that in the controls. In the extended evaluation, the incidence of renal tubule adenoma in 2,500 ppm males was significantly increased. The incidence of adenoma or carcinoma (combined) in 2,500 ppm males in the combined standard and extended evaluations were marginally increased over those in the controls. Male rats had an exposure-related increase in the severity of chronic nephropathy, which probably accounted for the reduced survival in the 1,300 and 2,500 ppm groups. The incidences of kidney cysts were increased in 1,300 and 2,500 ppm males. Hyperparathyroidism, secondary to the loss of renal function, was present in many exposed male rats. The incidences of parathyroid gland hyperplasia in all groups of exposed males were significantly greater than that in the controls.

2-YEAR STUDY IN MICE

Groups of 50 male and 50 female mice were exposed to dietary levels of 0, 300, 600, or 1,300 ppm primidone (equivalent to average daily doses of approximately 30, 65, or 150 mg/kg to males and 25, 50, or 100 mg/kg to females) in feed for 2 years.

Survival, Body Weights, Feed Consumption, and Clinical Findings

Survival of the 1,300 ppm males was significantly less than that of the controls. During the second year of the study, the mean body weights of 1,300 ppm male and female mice were less than those of the controls. The final mean body weights of 600 ppm males and females were less than those of the controls. Feed consumption by all exposed groups of mice was similar to that by the controls. During the latter part of the study, a treatment-related increase in the number of animals with swelling of the abdominal area was

observed; necropsy revealed that the swelling was due to liver nodules/masses.

Pathology Findings

The liver was a target organ in both male and female mice. The incidences and multiplicities of hepatocellular neoplasms (hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma) in all exposed groups of males and females (except hepatoblastoma in females) were significantly greater than those in the controls. The incidences of hepatocellular adenoma or carcinoma (combined) and hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) in all exposed groups exceeded the historical control ranges in 2-year NTP studies. The incidences of centrilobular hepatocyte hypertrophy were increased in exposed groups of males and females, and the severities increased with increasing exposure concentration. The incidences of cytoplasmic vacuolization were increased in all exposed groups of females and in 300 ppm males. Incidences of eosinophilic focus in all exposed groups of females were significantly greater than those in the controls.

Proliferative changes occurred in the thyroid gland in an exposure-related manner in male and female mice. Incidences of follicular cell hyperplasia were increased in all exposed groups of males and in 600 and 1,300 ppm females, but incidences of follicular cell adenomas were increased only in male mice.

GENETIC TOXICOLOGY

Primidone was mutagenic in *Salmonella typhimurium* strain TA1535 in the absence of S9 activation only; no mutagenicity was detected in strain TA98, TA100, or TA1537, with or without S9. Primidone did not induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells, with or without S9. The single *in vivo* study with primidone, a mouse bone marrow micronucleus test, also gave negative results.

CONCLUSIONS

Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity** of primidone in male F344/N rats based on a marginal increase in thyroid gland follicular cell neoplasms, primarily adenomas, and a marginal increase in renal tubule neoplasms. There was *no evidence of carcinogenic activity* of primidone in female F344/N rats exposed to 600, 1,300, or 2,500 ppm. There was *clear evidence of carcinogenic activity* of primidone in male B6C3F₁ mice based on the increased incidences of hepatocellular neoplasms, and the increased incidence of thyroid gland follicular cell adenomas was also considered to be chemical related. There was *clear evidence of carcinogenic activity* of primidone in female B6C3F₁ mice based on the increased incidences of hepatocellular neoplasms.

Exposure of rats to primidone resulted in increased incidences of hepatocyte cytoplasmic vacuolization and centrilobular hypertrophy in males and females and eosinophilic foci in females. The increased severity of nephropathy and increased incidence of renal tubule hyperplasia in male rats were related to primidone exposure. Exposure of male mice to primidone resulted in hepatocyte centrilobular hypertrophy and thyroid gland follicular cell hyperplasia. Exposure of female mice to primidone resulted in hepatocyte centrilobular hypertrophy and cytoplasmic vacuolization, eosinophilic focus, and thyroid gland follicular cell hyperplasia.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 13.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Primidone

	Male F344/N Rats	Female F344/N Rats	Male B6C3F ₁ Mice	Female B6C3F ₁ Mice
Concentrations in feed	0, 600, 1,300, or 2,500 ppm	0, 600, 1,300, or 2,500 ppm	0, 300, 600, or 1,300 ppm	0, 300, 600, or 1,300 ppm
Body weights	1,300 and 2,500 ppm groups less than the control group	1,300 and 2,500 ppm groups less than the control group	600 and 1,300 ppm groups less than the control group	600 and 1,300 ppm groups less than the control group
2-Year survival rates	13/50, 7/50, 4/50, 0/50	24/50, 27/50, 31/50, 28/50	35/50, 34/50, 31/50, 19/50	41/50, 42/50, 44/49, 39/50
Nonneoplastic effects	<u>Liver</u> : hepatocyte cytoplasmic vacuolization (26/50, 28/50, 33/50, 43/50); hepatocyte centrilobular hypertrophy (0/50, 14/50, 33/50, 40/50) <u>Kidney</u> : severity of nephropathy (2.2, 2.9, 3.4, 3.8); renal tubule hyperplasia (1/50, 2/50, 4/50, 10/50)	<u>Liver</u> : hepatocyte cytoplasmic vacuolization (25/50, 44/50, 46/50, 44/50); hepatocyte centrilobular hypertrophy (1/50, 36/50, 38/50, 35/50); eosinophilic focus (2/50, 0/50, 1/50, 18/50)	<u>Liver</u> : hepatocyte centrilobular hypertrophy (3/50, 30/50, 21/50, 18/50) <u>Thyroid gland</u> : follicular cell hyperplasia (8/49, 20/48, 31/50, 42/50)	<u>Liver</u> : hepatocyte centrilobular hypertrophy (1/50, 11/50, 11/49, 21/50); hepatocyte cytoplasmic vacuolization (3/50, 35/50, 39/49, 28/50); eosinophilic focus (8/50, 23/50, 24/49, 17/50) <u>Thyroid gland</u> : follicular cell hyperplasia (13/50, 12/48, 28/48, 49/50)
Neoplastic effects	None	None	<u>Liver</u> : hepatocellular adenoma (22/50, 41/50, 39/50, 32/50); hepatocellular carcinoma (12/50, 31/50, 35/50, 38/50); hepatocellular adenoma or carcinoma (31/50, 48/50, 47/50, 46/50); hepatoblastoma (0/50, 17/50, 26/50, 7/50); hepatocellular carcinoma or hepatoblastoma (12/50, 39/50, 40/50, 39/50); hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (31/50, 49/50, 49/50, 46/50) <u>Thyroid gland</u> : follicular cell adenoma (0/49, 3/48, 3/50, 6/50)	<u>Liver</u> : hepatocellular adenoma (15/50, 42/50, 45/49, 47/50); hepatocellular carcinoma (3/50, 11/50, 19/49, 38/50); hepatocellular adenoma or carcinoma (16/50, 42/50, 45/49, 50/50); hepatoblastoma (1/50, 4/50, 4/49, 4/50); hepatocellular carcinoma or hepatoblastoma (4/50, 12/50, 20/49, 39/50); hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (16/50, 42/50, 46/49, 50/50)
Uncertain findings	<u>Thyroid gland</u> : follicular cell adenoma (1/50, 1/50, 6/49, 3/49); follicular cell adenoma or carcinoma (2/50, 4/50, 7/49, 4/49) <u>Kidney</u> : renal tubule adenoma or carcinoma (standard and extended evaluations combined - 4/50, 2/50, 4/50, 7/50)	None	None	None
Level of evidence of carcinogenic activity	Equivocal evidence	No evidence	Clear evidence	Clear evidence

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Primidone

Genetic toxicology

<i>Salmonella typhimurium</i> gene mutations:	Positive in strain TA1535 without S9; negative in strains TA98, TA100, and TA1537 with and without S9
Sister chromatid exchanges	
Cultured Chinese hamster ovary cells <i>in vitro</i> :	Negative with and without S9
Chromosomal aberrations	
Cultured Chinese hamster ovary cells <i>in vitro</i> :	Negative with and without S9
Micronucleated erythrocytes	
Mouse bone marrow <i>in vivo</i> :	Negative

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on primidone on 12 December 1996 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

Gary P. Carlson, Ph.D., Chairperson
School of Health Sciences
Purdue University
West Lafayette, IN

Arnold L. Brown, M.D.
University of Wisconsin Medical School
Madison, WI

Thomas L. Goldsworthy, Ph.D., Principal Reviewer
Department of Experimental Pathology and Toxicology
Chemical Industry Institute of Toxicology
Research Triangle Park, NC

Robert LeBoeuf, Ph.D., Principal Reviewer
Corporate Professional and Regulatory Services
Human Safety Department
The Procter & Gamble Company
Cincinnati, OH

Janardan K. Reddy, M.D.
Department of Pathology
Northwestern University Medical School
Chicago, IL

Irma Russo, M.D.
Fox Chase Cancer Center
Philadelphia, PA

Louise Ryan, Ph.D., Principal Reviewer
Division of Biostatistics
Dana-Farber Cancer Institute
Boston, MA

Robert E. Taylor, M.D., Ph.D.
Department of Pharmacology
Howard University College of Medicine
Washington, DC

Frederick L. Tyson, Ph.D.
St. Mary's Hospital and Medical Center
Cancer Research Institute
Grand Junction, CO

Jerrold M. Ward, D.V.M., Ph.D.*
National Cancer Institute
Frederick, MD

* Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 12 December 1996, the draft Technical Report on the toxicology and carcinogenicity studies of primidone received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of primidone by discussing the uses of the chemical and the rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic lesions in male and female mice and nonneoplastic lesions in male and female rats and mice. The proposed conclusions for the 2-year studies in mice and rats were *equivocal evidence of carcinogenic activity* in male rats, *no evidence of carcinogenic activity* in female rats, *clear evidence of carcinogenic activity* in male mice, and *clear evidence of carcinogenic activity* in female mice.

Dr. Goldsworthy, a principal reviewer, agreed in principle with the proposed conclusions. He said the poor survival in 1,300 and 2,500 ppm male rats, as well as decreased weight gain, made the decision between *equivocal evidence* and *some evidence* unclear in male rats, even though the incidences of thyroid gland follicular cell and renal tubule neoplasms were above the historical control range. Dr. Goldsworthy asked whether it might have been appropriate with the male rat data to use the survival-adjusted "Poly-3" quantal response employed in the chloroprene study. Dr. J.K. Haseman, NIEHS, reported that the new "Poly-K" methods will be used routinely with the technical reports for the next review meeting. This and other newer methods have an advantage over current methods in that they do not require an assumption regarding whether a tumor is fatal or incidental. Dr. Goldsworthy thought that there was an overemphasis in the Introduction and Discussion sections on relating all of the neoplasm responses to a primary metabolite, phenobarbital, and that some discussion should be given to possible

carcinogenic activity of primidone and the other primary metabolite, phenylethylmalonamide.

Dr. Ryan, the second principal reviewer, agreed with the proposed conclusions. She liked the section dealing with plasma concentrations of primidone and phenobarbital and questioned whether markedly different plasma level patterns between rats and mice might explain differences in response between the species. Dr. Ryan noted the widespread human usage as an anticonvulsant and asked why some of these toxicology studies would not have been done as part of the FDA approval process. Dr. Dunnick said that primidone was developed in the 1950s and nominated because there were no long-term toxicology and carcinogenicity studies reported in the literature.

Dr. LeBoeuf, the third principal reviewer, agreed with the proposed conclusions. He commented that the pharmacokinetics and toxicokinetics, although limited in scope, were extremely useful for cross comparisons to studies with phenobarbital and, further, that this type of data should be collected routinely to aid in interpretation of other bioassays. Dr. LeBoeuf said that the confirmation of an absence of *Helicobacter* in this study was comforting with regard to interpretation of the neoplasm results in mice.

There was some discussion about the neoplasm-promoting activity of primidone/phenobarbital. Dr. J. Rice, International Agency for Research on Cancer, noted the markedly increased incidences of hepatoblastomas in exposed mice and said that agents capable of promoting hepatocarcinogenic effects in certain strains of mice, and especially in male mice, invariably generate a significant fraction of hepatoblastomas. This was consistently seen with phenobarbital. These neoplasms are highly malignant, metastasize readily, and are often lethal.

Dr. Goldsworthy moved that the Technical Report on primidone be accepted with revisions discussed and the conclusions as written. Dr. LeBoeuf seconded the motion, which was accepted unanimously with eight votes.

